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# Epidemiology, Production Losses, and Control Measures Associated with an Outbreak of Avian Influenza Subtype H7N2 in Pennsylvania (1996–98)

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SUMMARY. An outbreak of H7N2 low-pathogenicity (LP) avian influenza (AI) occurred in a two-county area in Pennsylvania from December of 1996 through April of 1998. The outbreak resulted in infection of 2,623,116 commercial birds on 25 premises encompassing 47 flocks. Twenty-one (one premise with infection twice) of the twenty-five infected premises housed egg-laying chickens and one premise each had turkeys, layer pullets, quail, and a mixed backyard dealer flock. Despite close proximity of infected flocks to commercial broiler flocks, no infected broilers were identified. Experimentally, when market age broilers were placed on an influenza-infected premise they seroconverted and developed oviduct lesions. The outbreak was believed to have originated from two separate introductions into commercial layer flocks from premises and by individuals dealing in sales of live fowl in the metropolitan New York and New Jersey live-bird markets. Source flocks for these markets are primarily in the northeast and mid-Atlantic areas, including Pennsylvania. Mixed fowl sold include ducks, geese, guinea hens, quail, chukar partridges, and a variety of chickens grown on perhaps hundreds of small farms. Infections with the H7N2 AI virus were associated with variable morbidity and temporary decreases in egg production ranging from 1.6% to 29.1% in commercial egg-laying chickens. Egg production losses averaged 4.0 weeks duration. Mortality ranged from 1.5 to 18.3 times normal (mean of 4.3 times normal). Duration of mortality ranged from 2 to 13 weeks (average of 3.9 weeks) in flocks not depopulated. Lesions observed were primarily oviducts filled with a mucous and white gelatinous exudates and atypical egg yolk peritonitis. Quarantine of premises and complete depopulation were the early measures employed in control of this outbreak. Epidemiological studies suggested that depopulation furthered the spread of influenza to nearby flocks. Thereafter, later control measures included quarantine, strict biosecurity, and controlled marketing of products.

RESUMEN. Epidemiología, pérdidas en la producción y medidas de control asociadas a un brote de influenza aviar del subtipo H7N2 en pensilvania (1996–1998).

Un brote de influenza aviar ocasionado por un virus de baja patogenicidad del tipo N7H2 se presentó en el área de Pensilvania entre los meses de Diciembre del 1996 y Abril del 1998, el cual resultó en la infección de 2,623,116 aves comerciales en 24 granjas y un total de 47 parvadas. Veinte de las veinticuatro granjas infectadas estaban dedicadas a la crianza de ponedoras comerciales (incluyendo una granja sospechosa) y una granja de pavos, una de levante de

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ponedoras, una de codornices y una granja de traspatio cría mixta de aves. A pesar de la proximidad de estas instalaciones infectadas a granjas de cría comercial de pollos de engorde, no se registraron brotes de la enfermedad en este tipo de aves. Cuando los pollos de engorde en edad de procesamiento fueron expuestos al virus de forma experimental, mediante el emplazamiento de los mismos en granjas previamente infectadas, los mismos presentaron seroconversión y desarrollaron lesiones en el oviducto. Se cree que el brote se originó debido a la introducción del virus en dos granjas de ponedoras comerciales por individuos asociados a la venta de aves en pie en los mercados locales de Nueva York y Nueva Jersey. Las aves vendidas en estos mercados provienen principalmente de las áreas del nordeste y Atlántico medio, incluyendo Pensilvania. Las especies aviares vendidas en estos mercados incluyen patos, gansos, gallinas de guinea, codornices, perdices y una variedad de gallinas criadas en cientos de granjas pequeñas. Las infecciones por el virus de influenza del tipo H7N2 presentaron diferentes grados de morbilidad y bajas temporales de la postura de un 1.6% a un 29.1% en ponedoras comerciales. Las bajas de la postura ocurrieron por un promedio de 4 semanas. La mortalidad observada fue de 1.5 a 18.3 veces más elevada que lo normal (para un promedio de mortalidad de 4.3 veces más alta de lo normal). La duración de los aumentos de la mortalidad observados fue de 2 a 13 semanas (para un promedio de 3.9 semanas) en granjas donde lo hubo despoblación de las parvadas. Las lesiones más comúnmente observadas fueron oviductos llenos de material mucoso y exudado gelatinoso blanco y peritonitis atípicas por retención de huevos. La cuarentena y despoblación de las granjas infectadas fueron las primeras medidas de control aplicadas para la erradicación de la enfermedad. Los estudios epidemiológicos realizados sugieren que la despoblación de las parvadas afectadas causó la diseminación de la enfermedad a parvadas cercanas. Otras medidas de control tomadas incluyeron la cuarentena, bioseguridad estricta y control del mercadeo de productos.

Key words: avian influenza, chickens, epidemiology, H7N2, H5N1, mice, serology

Abbreviations: AGID = agar gel immunodiffusion; AI = avian influenza; CAF = chorioallantoic fluid; ECE = embryonating chicken eggs; HA = hemagglutination; HP = high pathogenicity; LP = low pathogenicity; PADLS = Pennsylvania Animal Diagnostic Laboratory System; SPF = specific pathogen free; VTM = virus transfer medium

Since 1983 Pennsylvania has had three avian influenza (AI) epidemics. The first epidemic was in 1983–84 when over a period of 6 months (April–October 1983) an initially H5N2 low-pathogenicity (LP) AI virus became highly pathogenic (HP), resulting in the depopulation of over 16 million birds from 380 flocks in three states (6). The source of this initially LP H5N2 virus was not determined. Retrospective evidence suggests that the AI virus originated from the live-bird markets of New York, although it was not suspected as a source of AI virus, nor was testing initiated until 1986 (9). Following eradication of the 1983–84 H5N2 HPAI virus, Pennsylvania instituted a statewide AI-monitoring program in early 1985 that has tested over 140,000 samples yearly for the past 17 years from commercial poultry. In addition, more than 80,000 samples have been tested from flocks marketing birds to the live-bird markets.

The onset of the second AI epidemic in Pennsylvania occurred December 18, 1985, when 10-week-old roasters purchased for the New York live-bird markets were examined at the Pennsylvania State University Animal Diagnostic Laboratory and found to have H5N2 LPAI virus (5). The birds were showing relatively severe respiratory signs and experienced an increased mortality rate of 18%.

An immediate epidemiological investigation established that a poultry dealer had introduced the H5N2 LPAI virus responsible for the outbreak from the New York live-bird markets, presumably via contaminated crates. Because these flocks were being topped off (e.g., the heavier birds were being removed at 1 to 2 week intervals), this allowed the remaining susceptible birds to incubate any virus that was introduced and develop disease. When, for the first time, poultry in the live-bird markets were tested, many were found positive for the H5N2 LPAI virus associated with this outbreak. A statewide monitoring program provided assurance that influenza was not circulating undetected in other areas. This virus was introduced to an area of relatively low poultry flock density; the lower pathogenic characteristics of this virus and aggressive control and eradication efforts resulted in the virus being eradicated after depopulation of 350,000 broilers, roasters, and layers.

The third AI epidemic in Pennsylvania (1996–98) was associated with an H7N2 virus classified initially as LP. The pathological findings (primarily

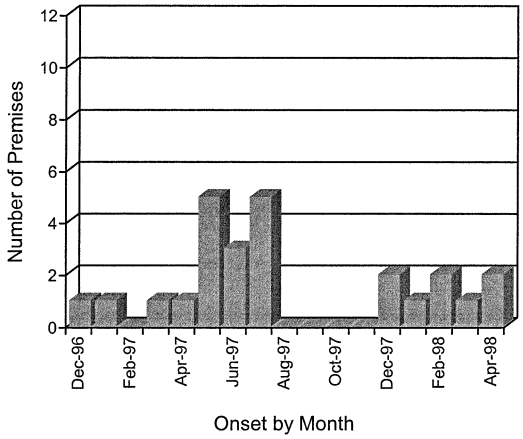


Fig. 1. Epidemic curve of disease onset of premises with an outbreak of AI subtype H7N2 infection in Pennsylvania (1996–98).

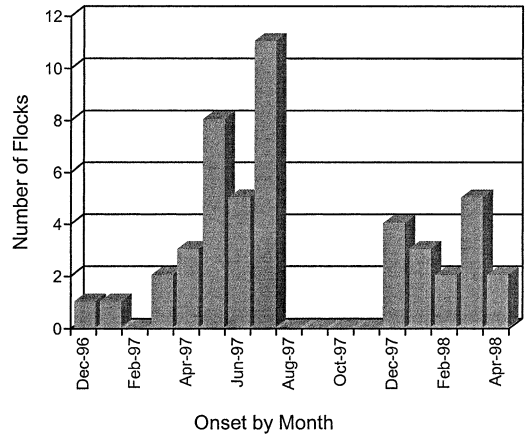


Fig. 2. Epidemic curve of disease onset of flocks with an outbreak of AI subtype H7N2 infection in Pennsylvania (1996–98).

a salpingitis with occasional oviduct necrosis) associated with the H7N2 virus involved in this epidemic have been described (14). Fluid, fibrin, and egg yolk material in the peritoneum (egg yolk peritonitis), as well as pulmonary congestion and pulmonary edema, were also frequently identified. Epidemiological evidence suggested that the source of the virus involved in this outbreak was the live-bird markets (3). The H7N2 has been consistently found in the live-bird markets since 1994. When tested in 2001, 49/81 (60%) of the New York and 12/28 (43%) of the New Jersey markets had chickens with AI virus.

It is also worthy of note that epidemiological observations made in the 1983–84 H5N2 epidemic and again in the initial cases of the 1996–98 H7N2 virus described in this paper suggested that depopulation programs may have in some situations been responsible for spread of the virus to nearby farms. Given these observations, a system of quarantines, strict biosecurity, controlled marketing, and repeated testing prior to quarantine release was instituted to control and successfully eradicate the H7N2 virus.

The epidemiology, production losses, and control measures associated with the 1996–98 H7N2 AI outbreak are described in this paper.

## MATERIALS AND METHODS

**Epidemiological investigation.** Investigations of flocks for AI virus resulted from laboratory samples submitted for poultry movement to live-bird markets in metropolitan New York (the source of the initial dealer infection), diagnostic samples from suspect flocks associated with nearby identified influenza positive flocks, and increased surveillance introduced in a 70-mile quarantined area. Growers had noted increased mortality and early egg production losses. Subsequently, diagnostic evaluations by the Pennsylvania Department of Agriculture and the Pennsylvania Animal Diagnostic Laboratory System (PADLS) confirmed new cases of AI. Oviduct lesions consisting of a white purulent material often accompanied by atypical egg yolk peritonitis were common and evident on field necropsies. Agar gel immunodiffusion (AGID) of serum collected at farms for antibodies against influenza, Directigen® Flu A test of cloacal and tracheal swabs for influenza viral antigen, and virus isolation in embryonated chicken eggs confirmed the case diagnosis of AI.

**Flock mortality.** Mortalities were calculated by taking an average of the mortality for each of the 4 weeks immediately prior to the onset of disease. Daily mortality counts were added for 7 days to determine weekly mortality counts. This average was divided into each weekly dead bird count beginning the day of disease onset and continuing. Where less than 7 days were available in any week of normal or disease mortality calculation, the figures were adjusted by using the average daily mortalities, respectively. Since within-flock specific mortality figures were used for the period immediately prior to the onset of increased mortality patterns, they are automatically age adjusted and represent a true picture of disease loss. Hence, mortalities were calculated on a weekly basis and averaged over the period of losses due to influenza or up to the time of depopulation. Average weekly mortality counts expressed in Tables 1 and 2 represent

Table 1. Mortality and egg production data for commercial chicken layer flocks depopulated or removed early, associated with an outbreak of AI subtype H7N2 infection in Pennsylvania (1996–98).

| | Mortality | | | | | Egg production | | |
|---|---|---|---|---|---|---|---|---|
| Premises No. Flock No. | Age (wk) | Avg. weekly normal | Avg. % weekly change | Avg. weekly times normal | Duration | Avg. weekly % change | Duration | Clinical disease onset |
| 2 | 44 | 0.04 | 0.14 | +3.7 | 4 wk | −6.1 | 4 wk | Jan. 16, 1997 |
| 4 (H1) | 30 | [A] | | +5.6 | 3 wk | Undetermined | Undetermined | Apr. 16, 1997 |
| 4 (H2) | 76 | | | +1.5 | 1 wk | Undetermined | Undetermined | Apr. 30, 1997 |
| 4 (H3) | 60 | | | +2.2 | 1 wk | Undetermined | Undetermined | Apr. 30, 1997 |
| 5 | 50 | | | +5.0 | 1 wk | Undetermined | Undetermined | May 08, 1997 |
| 6 (H1) | 70 | ns[B] | ns | ns | 1 wk | −7.3 | 1 wk | May 15, 1997 |
| 7 (H1) | 98 | | | +8.0 | 7 days | Undetermined | Undetermined | May 17, 1997 |
| 7 (H2) | 38 | | | +2.6 | 4 days | Undetermined | Undetermined | May 17, 1997 |
| 7 (H3) | 51 | | | +2.9 | 9 days | Undetermined | Undetermined | May 17, 1997 |
| 8 | 95 | 0.18 | 0.38 | +2.3 | 2 wk | Undetermined | Undetermined | May 28, 1997 |
| 9 | 103 | | | +2.9 | 1 wk | Undetermined | Undetermined | May 28, 1997 |
| 10 (H1) | 72 | 0.15 | 0.45 | +3.0 | 6 days | −4.1 | 6 days | Jun. 1, 1997 |
| 11 | 86 | | | +4.1 | 3 wk | −17.0 | 3 wk | Jun. 12, 1997 |
| 19 (H1) | 56 | | | +3.8 | 6 wk | −29.1 | 4 wk | Dec. 13, 1997 |
| 19 (H2) | 59 | | | +6.5 | 5 wk | −21.7 | 4 wk | Dec. 13, 1997 |
| 25 | 98 | ns | ns | ns | ns | Undetermined | Undetermined | Apr. 21, 1998 |

[A]Not calculated (blank spaces).
[B]No significant mortality.

increases times the average number of mortality prior to disease onset. For example, premise 2 had a weekly average mortality of 3.7 times normal for duration of 4 weeks (until depopulation). This flock of 127,300 hens averaged deaths losses of 50.5 birds per week for the 4 weeks prior to disease onset. For each of the 4 weeks following diagnosis of AI and before depopulation, the weekly bird losses were 81, 138, 257, and 191 (partial week, which was adjusted to weekly count). Similarly, average percent mortality for the 4 weeks prior to disease onset was 0.04%. For each of the 4 weeks before depopulation percent mortalities were 0.07%, 0.11%, 0.21%, and 0.15% (partial week). Summing these four percent mortality figures and dividing by four gives 0.14, the average percent change.

**Egg production.** Egg production losses were calculated with the same methods as flock mortality except percent production was used as a basis for averaging. In each case a 4-week average immediately prior to disease onset was compared with up to 4 weeks average production after infection onset. In a few young flocks where birds were approaching or in peak egg production (25 to 35 weeks), a smaller average normal egg production was used for a base comparison of either 1 or 2 weeks. This allowed for a better estimate of egg production losses as a result of influenza infection at a time when egg production would normally change quite rapidly. Since within-flock specific egg production figures were used for the period immediately prior to the onset of egg production loss patterns, they are automatically age adjusted and represent a true picture of egg production losses resulting from influenza infection.

**Sample collection.** During the 1996–98 AI outbreak in Pennsylvania, swab samples and field cases from AI suspect and quarantine flocks were collected for virus isolation and identification. Tracheal and cloacal swabs were placed in tubes containing 5 ml virus transfer medium (VTM) per tube, usually five swabs pooled per tube. Wet-drag swabs were obtained from cages, walls, and floors inside chicken houses as noted (7), with the exception of a media change; to each whirlpak bag 50 ml of viral transport media were used. Similarly, where surfaces were more appropriately sampled by hand swabbing, a minimum of five separate areas were swabbed with each sample. Field cases of sick or dead birds were submitted to animal diagnostic laboratories within PADLS, where tissue specimens of trachea, lung, air sac, sinuses, intestine, cecal tonsils, and oviduct were collected for virus isolation. Serum samples and fresh eggs were collected on a sampling basis for serological epidemiological surveillance.

**Virus isolation.** All swab samples and tissue specimens were processed for virus isolation using specific pathogen free (SPF) embryonating chicken eggs (ECE) following standard procedures (2,11). Briefly, tracheal and cloacal swabs within VTM in tubes were vortexed first, and then swabs were squeezed and removed. The swab samples were centrifuged at 1200 rpm for 10 min at 4°C, and the supernatants

Table 2. Mortality and egg production data for commercial chicken layer flocks maintained in production associated with an outbreak of AI subtype H7N2 infection in Pennsylvania (1996–98).

| | Mortality | | | | | Egg production | | |
|---|---|---|---|---|---|---|---|---|
| Premises No. Flock No. | Age (wk) | Avg. weekly normal | Avg. % weekly change | Avg. weekly times normal | Duration (weeks) | Avg. weekly % change | Duration (weeks) | Clinical disease onset |
| 12 (H1) | 74 | 0.06 | 0.91 | +14.3 | 4 | −3.4 | 4 | Jun. 26, 1997 |
| 12 (H2) | 74 | 0.05 | 0.88 | +18.3 | 4 | (+2.5) | 5 | Jun. 26, 1997 |
| 13 | 51 | 0.05 | 0.16 | +3.1 | 2 | −1.6 | 1 | Jul. 10, 1997 |
| 14 (H1) | 29 | 0.19 | 0.45 | +2.5 | 2 | −2.9 | 3 | Aug. 14, 1997 |
| 14 (H2) | 28 | 0.26 | 0.80 | +2.8 | 5 | Undetermined | Undetermined | Aug. 14, 1997 |
| 14 (H3) | 53 | 0.16 | 0.91 | +5.8 | 5 | −3.9 | 6 | Jul. 24, 1997 |
| 14 (H4) | 31 | 0.16 | 0.70 | +4.4 | 4 | −1.6 | 4 | Jul. 31, 1997 |
| 14 (H5) | 104 | 0.23 | 1.40 | +6.2 | 4 | −10.4 | 4 | Jul. 10, 1997 |
| 14 (H6) | 76 | 0.21 | 1.50 | +7.1 molted | 3 | −14.2 | 3 | Jul. 24, 1997 |
| 14 (H7) | 135 | 0.52 | 1.80 | +3.3 | 4 | −8.1 | 3 | Jul. 17, 1997 |
| 16 | 19 | ns[A] | ns | ns | ns | ns | ns | Jul. 11, 1997 |
| 17 (H1) | 89 | [B] | | +2.3 | 9 | Undetermined | Undetermined | Jul. 15, 1997 |
| 17 (H2) | 29 | | | +1.7 | 8 | Undetermined | Undetermined | Jul. 15, 1997 |
| 21 (H2) | 61 | | | +2.9 | 2.5 | −7.2 | 3.5 | Feb. 19, 1998 |
| 21 (H3) | 59 | | | +1.8 | 2.5 | −5.2 | 4.5 | Mar. 04, 1998 |
| 22 (H1) | 48 | 0.11 | 0.27 | +2.5 | 8 | −4.6 | 6 | Feb. 24, 1998 |
| 22 (H2) | 51 | 0.17 | 0.51 | +2.8 | 4 | −4.4 | 3 | Mar. 18, 1998 |
| 23 (H1) | 19 | | | ns | ns | ns | ns | Mar. 1998 |
| 23 (H2) | 55 | 0.12 | 0.25 | +2.1 | 13 | −6.1 | 13 | Mar. 10, 1998 |
| 23 (H3) | 43 | 0.10 | 0.18 | +1.7 | 10 | −4.3 | 11 | Apr. 22, 1998 |
| 23 (H4) | 71 | 0.08 | 0.23 | +3.1 | 4 | −5.6 | 6 | Apr. 22, 1998 |

were filtered through 0.45 μm syringe filters, or 10× antibiotics were added into each specimen and incubated at room temperature for 30–60 min without filtration. Tissue specimens were diluted with VTM at a dilution of 1:5 to 1:10 (w/v) and then were placed in a stomacher bag and homogenized in a stomacher blender for 3 to 5 min. The supernatant was then transferred to a centrifuge tube followed by centrifugation and filtration. Each specimen was inoculated into 9–11-day-old SPF ECE via chorioallantoic cavity route, 0.2 ml per egg, five eggs per specimen. After 72 to 96 hr of incubation at 37°5 C in an egg incubator, the inoculated ECE were removed and chilled at 4°C for a minimum of 4 hr or overnight. Chorioallantoic fluid (CAF) was then harvested from dead (>24 hr) and alive ECE of each specimen. The CAF was screened for the presence of AI virus by hemagglutination (HA) test (9). Two serial ECE passages were accomplished before calling a specimen negative for avian influenza. CAF with hemagglutinating activity was assayed by a commercial Directigen® Flu A test (13) and also by hemagglutination-inhibition (HI) test (13) using reference antisera to identify the virus as either AI virus or Newcastle disease virus (NDV), respectively. All HA-positive isolates were sent to NVSL for confirmation of virus identification and serotyping of AI viral hemagglutination and neuraminidase subtypes.

**Wild-caught mice.** House mice, *Mus musculus*, were collected from 10 poultry premises comprising 18 houses between June and September 1997. Mice were collected in Tin Cat® live catch mice traps, humanely euthanatized, and stored at –70°C until processing. In total 141 mice were collected, from which 46 intestine pools and 46 lung tissue pools were harvested and stored at –70°C. In addition, one lung and one intestine pool from two European starlings (*Sternus vulgaris*) captured in a single house were evaluated. Mice were collected during the early stages of the influenza outbreak for each poultry flock. Virus isolations used standard methodology (11). Two embryonating chicken egg passages were completed before calling samples negative.

**Agar gel immunodiffusion test for antibody detection.** Agar gel immunodiffusion (AGID) test was used to detect antibodies to AI virus in chicken sera and egg yolks (1,12). Individual serum and egg samples were tested.

**Experimental study in mice.** Groups of eight BALB/c and CAST/Ei mice were anesthetized with

Table 3. Sources of flock exposure for an outbreak of AI subtype H7N2 infection in Pennsylvania 1996–98).

| | |
|---|---|
| Area spread[A] | 9 premises |
| Association with bird depopulation | 6 premises |
| Association with live bird markets | 3 premises |
| Manure spread | 3 premises |
| Unknown | 2 premises |
| Movement out of quarantine zone | 1 premise |
| Equipment contact | 1 premise |
| Total | 25 premises |

[A]Re-emergence of the original infection or introduction of a second H7N2 virus.

inhaled metafane and inoculated intranasally with $10^6$ mean infectious dose ($EID_{50}$) of LPAI viruses A/chicken/Pennsylvania/11767-1/97 (H7N2), A/chicken/Pennsylvania/19241/97 (H7N2), or HPAI virus A/Hong Kong/156/97 (H5N1). Groups of two BALB/c and CAST/Ei mice were similarly inoculated with normal allantoic fluid (Sham). Three mice were euthanatized with pentobarbital (100 mg/kg) and necropsied on day 4 postinoculation (PI). Selected tissues were taken for routine histopathology. Virus isolation was attempted on trachea and lungs from two mice of each group. Previously, BALB/c mice were shown to be *Mx1*− genotype and susceptible to laboratory infection to human influenza viruses, while CAST/Ei mice were *Mx1*+ genotype and resistant to laboratory infection with human influenza virus strains (4).

## RESULTS

**Epidemiology of flock exposures sources.** Table 3 depicts the estimated source of flock exposures associated with the Pennsylvania outbreak of H7N2 LPAI virus. Association with the New York and New Jersey live-bird markets was the probable source of introduction of AI virus in the first case, December 1996. The owner maintained approximately 50 mixed fowl on site, which were used to fill orders for specialty birds and poultry. The main portion of the business was picking up birds at separate farms over several counties and transporting them to the dealer premise for some limited reassortment and direct shipment to the markets. While trace back investigations from the original infected birds provided by the New York Department of Food and Agriculture were completed by Pennsylvania Department of Agriculture personnel, it was determined that this dealer visited a total of 405 farms (premises) during the immediate 3-month period prior to the outbreak (October to December 1996). The second case was also in Lebanon county and a distance of 1.5 miles from the dealer premise. The third case of AI virus infection was associated with the owner of two small layer flocks (one brown and one white). Both houses contained hens, which were sick with respiratory signs for 2 and 3 weeks, respectively, for the white and brown layers. The white layers had mortality of 10.7 times normal for 1 of 2 weeks of infection. Both flocks had four dealers from both Pennsylvania and New York making multiple loadouts from each flock while clinical signs were apparent in the poultry. No birds were taken to the PADLS during this time, making the suspected diagnosis of AI impossible to confirm. Manure was removed from these chicken houses during the period when it was likely to be infectious and spread on a dairy (about 1.25 miles away) near the first confirmed case of AI in Lancaster county (premise 4) with an onset of clinical disease of April 14, 1997. From there bird depopulation was the most significant source of spread of the influenza virus for the next series of cases in the area through July of 1997.

**Serological epidemiology (serum).** The presence of AGID antibodies to AI virus was determined in sera collected from random birds on 34 of 47 flocks (Table 4). Twenty-four flocks had antibodies present in greater than 50% of the birds, with 10 flocks having antibodies in 50% or less of the selected birds. Multiple serum antibody measurements were taken temporally in 17 flocks, of which 11 had decreasing serum antibody. Five flocks had rapid and steep decreasing detected antibody. On four of five flocks (premise 14 [H3, H4, H5, and H6]) the declines occurred over a 4 to 5 month period. Flock 13 experienced a strong detectable antibody decline in 1 month. Six flocks (premises 14 [H2], 16, 22 [H1], and 23 [H1, H2, and H3]) had increasing antibody presence or remained constant for periods of 5–20 months. Flock 16 maintained antibody present in 20 of 29 birds selected (69%) a full 20 months following two molting periods until age 137 weeks, when the flock was processed as spent hens. This flock's origin was flock 15, where all of 20 pullets selected (July 23, 1997) had measurable antibody at 21 weeks of age. Flock 23 (H1) had an apparent increase in the number of birds with measurable antibody to a maximum of 50% of birds selected. Interestingly, this same flock also had 30 randomly placed sentinel hens to seroconvert. Three of the four flocks in complex premise 23 experienced constant or increasing antibodies over time, with flock 23 (H4) only experiencing a slight decrease. Similarly, sentinel

Table 4. Premises and test dates for serological and egg yolk epidemiology of flocks with an outbreak of AI subtype H7N2 infection in Pennsylvania (1996–98).

| Premises | House | Clinical disease onset | Serology | | Other |
|---|---|---|---|---|---|
| | | | Serum (test date) | Egg yolk (test date) | |
| 1 | Poultry dealer premises | Dec. 6, 1996 | —[A] | — | Environment H7N2 AI virus+ (Dec. 6, 1996), H6N8 from duck tracheal swabs, birds reported clinically normal (Lebanon County) |
| 2 | Single house | Jan. 16, 1997 | 15/20, 75% (Feb. 3, 1997) | — | (Lebanon County) |
| 3 | H1,[B] H2 | Mar. 13, 1997 | — | — | Lancaster County (suspect) |
| 4 | H1 | Apr. 16, 1997 | 3/30, 10% (Apr. 18, 1997); 11/20, 55% (May 3, 1997) | — | — |
| 4 | H2 | Apr. 30, 1997 | 13/30, 43% (May 6, 1997) | — | — |
| 4 | H3 | Apr. 30, 1997 | 10/30, 33% (May 6, 1997) | — | — |
| 5 | Single house | May 8, 1997 | — | — | Immediately across road from suspect farm 3 (about 200 m), mortality pattern suggestive of AI |
| 6 | H1, H2 | May 15, 1997 | H1, 1/5, 20% (May 21, 1997) | — | — |
| 7 | H1, H2, H3 | May 17, 1997 | H3, 4/20, 25% (May 19, 1997) | — | H1, seven mortality birds with clinical lesions of AI (May 22, 1997) |
| 8 | Single house | May 28, 1997 | 2/25, 8% (Jun. 4, 1997) | — | Tracheal, lung, gut, and oviduct pooled tissues AI virus+ (Jun. 4, 1997); 1/20, 5% (Jul. 30, 1997) post C&D environments AI virus+ |
| 9 | Single house | May 28, 1997 | 2/20, 10% (Jun. 4, 1997) | — | — |
| 10 | H1, H2 | Jun. 1, 1997 | H1, 1/25, 4% (Jun. 4, 1997); H2, 0/20, 0% (Jun. 4, 1997) | — | — |
| 11 | Single house | Jun. 12, 1997 | 9/13, 69% (Jun. 25, 1997) | 15/30 50% (Jun. 30, 1997) | — |
| 12 | H1 | Jun. 26, 1997 | 28/30, 93% (Jul. 15, 1997); 17/30, 57% (Sep. 19, 1997); 14/30, 47% (Oct. 15, 1997) | 29/30, 97% (Jul. 23, 1997); 3/30, 10% (Sep. 10, 1997); 0/30, 0% (Oct. 15, 1997) | 2/20 environments AI virus+ (Jul. 17, 1997) |
| 12 | H2 | Jun. 26, 1997 | 30/30, 100% (Jul. 15, 1997); 19/30, 63% (Sep. 19, 1997); 14/30, 47% (Oct. 15, 1997) | 28/30, 93% (Jul. 23, 1997); 2/30, 6.7% (Sep. 19, 1997); 0/30, 0% (Oct. 15, 1997) | 2/20 environments AI virus+ (Jul. 17, 1997) |
| 13 | Single house | Jul. 10, 1997 | 3/30, 10% (Aug. 26, 1997); 19/20, 95% (Sep. 15, 1997); 6/19, 32% (Nov. 21, 1997) | 0/30, 0% (Sep. 8, 1997); 39/51, 76% (Sep. 24, 1997); 7/60, 12% (Nov. 7, 1997) | — |

Table 4. Continued.

| Premises | House | Clinical disease onset | Serology | | Other |
|---|---|---|---|---|---|
| | | | Serum (test date) | Egg yolk (test date) | |
| 14 | H1 | Aug. 14, 1997 | 12/20, 60% (Aug. 22, 1997); 8/20, 40% (Jan. 9, 1998) | — | — |
| 14 | H2 | Aug. 14, 1997 | 5/20, 25% (Aug. 25, 1997); 11/20, 55% (Jan. 9, 1998) | — | — |
| 14 | H3 | Jul. 24, 1997 | 19/20, 19% (Sep. 8, 1997); 4/20, 20% (Jan. 9, 1998) | — | — |
| 14 | H4 | Jul. 31, 1997 | 9/20, 45% (Aug. 13, 1997); 18/20, 90% (Sep. 8, 1997); 6/20, 30% (Jan. 9, 1998) | — | — |
| 14 | H5 | Jul. 10, 1997 | 4/20, 20% (Jul. 11, 1997); 9/20, 45% (Oct. 31, 1997); 0/20, 0% (Jan. 9, 1998) | 3/20, 15% (Jul. 14, 1997) | Index flock this farm; 3/20 AI virus+ from mortality sample (Jul. 14, 1997) |
| 14 | H6 | Jul. 24, 1997 | 7/10, 70% (Jul. 28, 1997); 3/20, 15% (Jan. 9, 1998) | — | — |
| 14 | H7 | Jul. 17, 1997 | 17/20, 85% (Sep. 8, 1997) | — | — |
| 15 | Single house | Jul. 10, 1997 | 0/20, 0% (Jun. 26, 1997); 20/20, 100% (Jul. 23, 1997) | — | 17-week-old pullets; 1/20 tracheal swabs AI virus+ (Jul. 10, 1997) |
| 16 | Single house | Jul. 11, 1997 | 9/19, 47% (Jul. 14, 1997); 20/29, 69% (Apr. 29, 1999) | — | Received pullets from farm 15 |
| 17 | H1 | Jul. 15, 1997 | 6/30, 20% (Sep. 2, 1997); 9/30, 30% (Oct. 22, 1997) | 9/30, 30% (Sep. 2, 1997) | — |
| 17 | H2 | Jul. 15, 1997 | — | 4/29, 14% (Jul. 27, 1997); 1/30, 3% (Sep. 2, 1997) | — |
| 18 | H1, H2 | Dec. 8, 1997 | 6/10, 60% (Dec. 9, 1997); 30/30, 100% (Dec. 12, 1997) all samples from 9.5-week-old birds | — | Turkeys—clinically ill with respiratory signs; AI virus+ from 6/8 trachea/sinus swabs |
| 19 | H1, H2 | Dec. 13, 1997 | H1, 1/4, 25% (Dec. 18, 1997) | — | H1, 2/4 sera MG+; H2, 1/4 tissue H7N2 AI virus+ (Dec. 18, 1997) |
| 20 | Complex 14 | Jan. 5, 1998 | — | — | Reemergence of H7N2 infection in farm 14 in 3 houses |
| 21 | H1 | Feb. 19, 1998 | 11/20, 55% (Feb. 26, 1998); 15/30, 50% (Apr. 28, 1998) | — | 30 sentinel hens placed shortly after infection diagnosed with no serologic conversion noted |

Table 4. Continued.

| Premises | House | Clinical disease onset | Serology | | Other |
|---|---|---|---|---|---|
| | | | Serum (test date) | Egg yolk (test date) | |
| 21 | H2 | Mar. 4, 1998 | 21/30, 70% (Mar. 11, 1998); 22/30, 73% (Apr. 28, 1998) | — | 30 sentinel hens placed shortly after infection diagnosed with no serologic conversion noted |
| 22 | H1 | Feb. 24, 1998 | 15/20, 75% (Feb. 25, 1998); 12/20, 60% (Jul. 9, 1998) | — | H7N2 isolated from lung/tracheal intestine and oviduct (Feb. 25, 1998) |
| | H2 | Mar. 18, 1998 | 11/19, 58% (Apr. 10, 1998); 9/20, 45% (Jul. 9, 1998) | — | — |
| 23 | H1 | Mar. 1998 | 1/20, 5% (Apr. 24, 1998); 16/32, 50% (Apr. 22, 1999) | 0/24, 0% (Mar. 25, 1998) | 30 sentinel hens placed (Jul. 17, 1998); sentinel serology, 0/30, 0% (Sep. 4, 1998); 9/30, 30% (May 3, 1999) |
| 23 | H2 | Mar. 10, 1998 | 2/20, 10% (Mar. 19, 1998); 19/20, 95% (Jul. 17, 1998) | 2/24, 8.3% (Feb. 98); 0/20, 0% (Mar. 14, 1998); 3/24, 12.5% (Mar. 25, 1998) | 30 sentinel hens placed (Jul. 17, 1998); sentinel serology 0/29, 0% (Aug. 14, 1998) |
| 23 | H3 | Apr. 22, 1998 | 1/20, 5% (Apr. 24, 1998); 15/30, 50% (Jul. 17, 1998) | 0/20, 0% (Mar. 14, 1998) 0/24, 0% (Mar. 25, 1998) | 30 sentinel hens placed (Jul. 17, 1998); sentinel serology 12/28, 43% (May 3, 1999) |
| 23 | H4 | Apr. 22, 1998 | 2/25, 8% (Apr. 24, 1998); 16/20, 80% (Jun. 9, 1998); 17/30, 57% (Apr. 22, 1999) | 0/20, 0% (Mar. 14, 1998) 0/24, 0% (Mar. 25, 1998) | 30 sentinel hens placed (Jul. 17, 1998); sentinel serology 0/30, 0% (May 3, 1999) |
| 24 | H1 | Apr. 14, 1998 | 9/10, 90% (Apr. 14, 1998); 22/25, 88% (Apr. 21, 1998) | — | Quail; H7N2 AI virus from 5/6 tracheal pools of 30 birds total |
| 25 | H1 | Apr. 21, 1998 | 2/10, 20% (Apr. 21, 1998); 28/60, 47% (May 13, 1998) | — | — |

[A]— = No data available.
[B]H1, H2, etc. = house 1, house 2, etc.

Table 5. Mortality and virus isolation data from an experimental study with AI viruses in BALB/c and CAST/Ei mice.

| | Mouse strain BALB/c | | | Mouse strain CAST/Ei | | |
|---|---|---|---|---|---|---|
| | | Virus isolation[B] | | | Virus isolation[B] | |
| Groups | Mortality[A] | Trachea | Lung | Mortality[A] | Trachea | Lung |
| Control | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| PA/11767/97 (H7N2) | 0/5 | 1/2 ($10^{2.5}$) | 0/2 | 0/5 | 0/2 | 0/2 |
| PA/19241/97 (H7N2) | 0/5 | 2/2 ($10^{6.2}$) | 2/2 ($10^{5.2}$) | 0/5 | 0/2 | 0/2 |
| HK/156/97 (H5N1) | 5/5 | 2/2 ($10^{6.0}$) | 2/2 ($10^{7.8}$) | 5/5 | 2/2 ($10^{4.0}$) | 2/2 ($10^{7.2}$) |

[A]Number of deaths/total mice observed for 10-day period.
[B]Number of positive mice/number of mice with virus isolation attempts (titer of AI virus isolated in $EID_{50}$).

hens also converted to positive antibody status in flock 23 (H3). There was some anecdotal evidence that this complex may have experienced a second and separate introduction of H7N2 influenza A virus challenge. High frequency of antibody positive birds was identified in one of the few nonlayer hen flocks: in flock 24, a small quail flock of 300 birds housed in a garage, 9 of 10 (90%) and 22 of 25 (88%) of birds had antibody when sampled early in the infection, 1 week apart.

**Serological epidemiology (egg yolk).** Egg samples were collected from nine flocks. In three flocks (premise 12 [H1 and H2] and 13), multiple collections allowed for some assessment of antibody response as measured by the agar gel immunodiffusion test (Table 4). Generally, there was a larger percentage of eggs with detectable antibody when eggs were collected early in the disease outbreak. This usually mirrored similar levels in serum from birds taken at the same time. In both flocks 12 (H1) and (H2) in a period of 6 weeks, measurable antibody decreased rapidly from 97% and 93% to 10% and 20%, respectively, in eggs sampled. In each case serum antibody levels were still well above 50% of hens sampled at 6 weeks. By 11 weeks no detectable antibody was present from a sample of 30 eggs, while serum antibody on a flock basis was still detected at 47% of chickens sampled. Similarly, flock 13 had a drop in seropositive egg yolks from 76% to 12%. Serum detectable antibody on a flock basis dropped from 95% to 32% during a similar time interval.

**Mortality and egg production data for layer flocks depopulated or removed early.** Good records were available on 11 premises comprising 16 flocks where flock mortality and egg production data were determined. These flocks were depopulated early and data were analyzed up to the time of depopulation (Table 1). Two of sixteen flocks reported no significant mortality changes. Twelve of sixteen flocks experienced average egg production losses ranging from –4.1% to –29.1%. The duration of egg production loss ranged from 6 days to 3 weeks. The flock averaging –17% loss over 3 weeks had a single weeks' egg production loss of –33.4% (premises 11). This flock experienced the most severe clinical signs of all flocks investigated. Mortality increases above normal amounts were noted in 14 of 16 flocks. Mortality losses above the normal daily mortality ranged from 4 days to 6 weeks. Increased average mortality varied from 1.5 to 8.0 times normal rates. Only a single flock (premise 4 [H2]) had less than 2.2 times normal mortality, with the exception of two flocks, which had no significant mortality. Six flocks had mortality rates in the 2.2 to 3.0 times normal range. Seven flocks had over 3.0 times normal mortality rate.

**Mortality and egg production data for layer flocks remaining in normal production.** Eight premises comprising 21 flocks remained in production for normal flock cycles (Table 2), where egg production and mortality data could be determined based on good records. Three of twenty-one flocks had insufficient records to determine egg production losses. Two of twenty flocks had no noted egg production losses, one of which received infected pullets but never experienced any detectable production losses (premise 16) despite being molted two times. A single flock had an average increase of 2.5% egg production (premise 12 [H2]).

Egg production losses occurred from 1 to 13 weeks in 15 flocks, and such losses ranged from –1.6% to –14.2%. Eight flocks had egg production losses ranging from –1.6% to –4.6%. Seven flocks had egg production losses equal to or greater than –5.2%. Increased hen mortalities were observed from 2 to 13 weeks in the 19 of 21 flocks that had

above normal mortality. Mortality increases ranged from 1.7 to 18.3 times normal mortality rates. Two flocks (premise 12 [H1 and H2]) had individual weekly mortality rates 32 times greater than normal (data not shown). Eleven of nineteen flocks had mortality rates ranging from 2.1 to 4.4 times normal. One flock (premise 23 [H1]) had no significant (ns) mortality losses. Three flocks had increased mortality rates below 2.0 times normal. Five flocks had mortality rates ranging from 5.8 to 18.3 times normal.

**Studies in mice.** All intestine and lung pools from wild-caught mice from the farms containing AI virus infected poultry were negative for AI virus. No influenza virus was isolated from lung or intestinal samples from two European starlings.

In the experimental studies, the inoculation of laboratory mouse strains with LP H7N2 Pennsylvania AI viruses did not result in clinical signs or death (Table 5). The HP Hong Kong H5N1 AI virus produced weight loss, lassitude, ruffled fur, and death in all mice (Table 5). However, the mean death time was shorter in CAST/Ei (5.2 days) than BALB/c (7.0 days) mice. LPAI challenge virus was not recovered from tracheas and lungs of the influenza resistant CAST/Ei mice, but LPAI virus was isolated from influenza-susceptible BALB/c mice. The titers were highest in mice inoculated with A/chicken/Pennsylvania/19241/97 (H7N2). By contrast, the HP Hong Kong H5N1 AI virus replicated to moderate to high titers in tracheas and lungs of both BALB/c and CAST/Ei mice. However, the virus titers in the tracheas were lower in CAST/Ei than in the BALB/c mice.

Histologically, all mice inoculated with normal allantoic fluid (sham) or A/chicken/Pennsylvania/11967-1/97 (H7N2) lacked lesions, and AI viral antigen was not detected in tissues of the respiratory tract, except in one AI virus-inoculated mouse that had mild peribronchial cuffs of lymphocytes. One BALB/c mouse inoculated with A/chicken/Pennsylvania/19241/97 (H7N2) had mild focal pneumonitis with necrotizing bronchitis and tracheitis with AI viral antigen in some respiratory epithelium of bronchi and trachea. For the CAST/Ei mice, only one had lesions and they were mild focal bronchitis with rare AI viral antigen in the respiratory epithelium. By contrast, the HK/156 H5N1 AI virus produced severe necrotizing bronchitis and alveolitis in all mice of both strains, and AI viral antigen was common in respiratory epithelium and alveolar macrophages. Necrotizing tracheitis and rhinitis were present, and AI viral antigen was demonstrated in respiratory epithelium.

## DISCUSSION

Area spread (Table 3) was responsible for nine cases of influenza and six were associated with bird depopulation. Area spread is a comprehensive term that includes unknown biosecurity breeches and other traditional farm and community practices that can result in movement of infectious agents. Depopulating laying hens humanely was accomplished by removing the hens from cages and placing them into 30 yard or larger metal waste dumpsters covered with vinyl tarps and filled with $CO_2$ gas. These birds were transported to a landfill with disinfected vehicles and closely monitored for either leakage or feather loss. Neither of these potential sources of spread was problematic. Eggs on depopulated flocks were sent to the landfill or in a few cases buried on site with poultry. These methods involve using many persons for large commercial modern hen houses in the depopulation and disposal process. In the process, much organic debris, including feathers, dust, and fecal material, was stirred up and became airborne. Ironically, in some situations, new farms were diagnosed with AI within a few days of depopulating AI-infected chickens on laying facilities located within 1 to 1.25 miles. The pattern of the grower finding dead hens in cages, often with heads stretched out in the feed troughs and occasionally accompanied by mild respiratory noises, was common. Attempts to construct wooden frameworks around fan housings with landscape nursery cloth attached did not effectively contain the apparent airborne virus spread, and hence depopulation of flocks was abruptly discontinued. Measures used to determine when individual farm quarantines could be released and poultry products moved were allowing the flocks to remain in production and monitoring the infection progression through surveillance of mortality cultures, serology, egg yolk antibody, and environmental culture, plus later sentinel hen surveillance. Nest run eggs were held in farm coolers during the early stages of influenza infection and then permitted to be sent to further processing plants (breakers) provided dedicated trucks and a closed loop of egg handling and transport materials was ensured. Eggs were processed on the last day of the working week and scheduled for the final flock(s) of the day. Special cleaning measures were placed on materials.

Avian influenza reemerged in December 1997/ January 1998 on premise 20 (original infection on premise 14). Hence, of 25 total premises, one premise that still contained resident flocks from the original infection had influenza isolated a second time (i.e., premise 14 and premise 20 are the same site). The onset of the clinically detected infection resulted from diagnostic work-up of 10 normal daily mortality hens. Avian influenza virus was isolated from one each of five cloacal and tracheal swab pools and two oviduct swab pools. In total, three of the six houses with hens broke with infection on this reemergence. This laying hen complex consisted of seven houses, of which six houses maintained flocks, for a total of approximately 490,000 laying hens. Five of the six houses [original references 14(1), (2), (3), (4), (6)] housed flocks that were present at the onset of clinical disease on July 10, 1997. The original house 14(7) was depopulated on September 15, 1997, and birds were transported to a landfill. This house was not restocked until October of 1998. Mortality and cloacal swabs of random selected hens were monitored through September 8, 1997.

House 14(5) was replaced with pullets on December 16, 1997, which were serologically monitored on January 9, 1998 with the AGID test, and no antibody was detected from 20 randomly selected birds.

Premise 14 was released from quarantine on November 26, 1997, on determination of no clinical sings of avian influenza and two environmental tests and cloacal swabs of birds at about 30 days apart. Between 14 and 20 environmental samples were taken from each of seven houses on October 6, 1997 and November 10, 1997. From the initial index case on July 11, 1997 (house 5), seven samples in three houses (2, 5, and 6) were positive for type A influenza, of which influenza virus was isolated in house 5 from three samples. Additionally, 262 normal mortality birds were evaluated for AI virus by the Directigen Flu A test and embryo inoculation for virus isolation. Specifically, the number of individual house mortality samples for premise 14 during this period was as follows: (1) 31, (2) 32, (3) 32, (4) 31, (5) 70, (6) 46, and (7) 16. A sample size of 30 was selected with a 95% confidence of obtaining a positive sample if 10% of the birds were shedding detectable influenza virus. These targeted samplings were spread out over a period along with the random cloacal swab evaluations of a total of 772 birds from the seven houses in the complex in efforts of detecting any latent infections. Sampling in house five (70 mortality birds) was more comprehensive since this was the index case.

Despite extensive testing of random and normal mortality birds, this inapparent or latent influenza virus was not noted until later in the disease process. The only other plausible explanation would be the possibility of a second introduction of the H7N2 avian influenza virus in which there was incomplete antibody protection allowing for the pathologic effects of the virus noted. Similarly, these flocks did experience mortality and egg production losses not unlike those losses previously described in this outbreak (data not shown for re-emergence or second infectious challenge). This detection failure led to the presumed contamination of a two-house premise [19 (H1) (H2)] through the spread of manure and/or vehicle contact. Re-evaluation of the methods employed for releasing quarantined flocks was reviewed. Subsequently, 30 susceptible hens (influenza negative) were placed randomly in several weight cages throughout the houses. Four other infected premises, which maintained hens in production, similarly, had 30 sentinel hens placed. Where possible some of these hens were placed in the manure pit or in manure storage and drying sheds receiving direct manure movements from previously infected premises. Sentinel hens were monitored serologically at appropriate intervals. Biosecurity may have prevented this exposure and subsequent infection of the farm 19 premise.

Extensive biosecurity measures become increasingly difficult to sustain in areas of high poultry densities (Lancaster County, PA) and where infectious agents can be spread through manure movement. Lengthy disease outbreaks provide added challenges and stress on individual flock owners, outside electric and maintenance contractors, feed and supply delivery agents, service personnel, cleaning and disinfection crews, government agencies, and others. However, the necessity for maintaining strict control and risk reduction measures was apparent. Without stringent standards for manure movement, the industry risks possible exposures of completely immunologically naive poultry populations outside of a quarantined area. However, manure restrictions are probably needed for less than 30 days following complete bird removal from a premise. Several thousand manure drag swab samples from selected houses were taken throughout flock production until other diagnostic tests determined that the flocks appeared to have recovered from influenza, and a positive sample was

noted with perhaps only three individual samples on separate samplings. The positive samples were found relatively soon after flocks became infected with the influenza virus. By contrast, in the 1983–84 Pennsylvania H5N2 HPAI outbreak, influenza virus was reported to be isolated from manure for periods beyond 30 days. This was not the Pennsylvania experience with the H7N2 LPAI. The host for the avian influenza virus is the poultry—not the environment.

Of flocks that were depopulated early and quality records were available for analysis, 12 of 16 flocks (75%) experienced egg production losses ranging from –4.1% to –29.1% (Table 1). One of the flocks (premise 11) experienced a significant decrease in egg production of –33.4% and a weekly mortality increase of 7.6 times the normal average for the week of depopulation (data not shown). This was the most significant clinically infected flock in terms of morbidity. With these flocks, none remained in production long enough to fully determine the extent of production losses. Flocks 19 (H1 and H2) remained in production the longest of any flocks depopulated early, 6 and 5 weeks, respectively. The weekly mortalities dropped below 2.0% the last several weeks prior to depopulation; however, the egg production losses continued above 15% (data not shown). Where flocks were maintained in production and mortality and egg production losses could be further analyzed, seven of eighteen flocks (39%) experienced egg losses of equal to or greater than –5.2% (Table 2). The duration of egg production losses for these flocks ranged from 3 to 13 weeks [flock 23 (H2)], with an average of 5.3 weeks loss. Nineteen of twenty-one flocks (90%) had increased mortalities ranging from 1.7 to 21.3 times normal. Flocks 12 (H1), (H2) had some very high record mortalities; however, these data are skewed somewhat by several weeks of very hot weather. While weather and other stress factors no doubt effect both egg production and mortality in commercial layer flocks, no other primary respiratory pathogen or other infectious agents were known to directly contribute to the flocks' health, excluding secondary *E. coli* infections that occurred and likely contributed to the egg yolk peritonitis observed grossly (fibrin flecks in abdominal cavity). In addition this outbreak, consisting of 25 premises and 47 flocks, occurred over all four seasons. Hence, both seasonal variation and multiple ages associated with 47 separate flocks reduce the likelihood that these variables caused by either of these factors had a significant impact on this population. Hence, the flocks' mortality and egg production losses appeared to be at least in large portion resultant from this H7N2 infection.

The detection of antibody was variable among flocks. Eleven of seventeen flocks (64.7%) had decreasing antibodies detected (Table 4). Of the six flocks with increasing antibody detection, flock 16 maintained a significant level of chickens (69%) with measurable antibody for 20 months (April 29, 1999), at which time this flock was 113 weeks of age. Hence, this LP influenza virus did not result in full conversion of birds (100%) with detectable antibody, many of which experienced declining antibodies on a flock basis. This allowed monitoring of individual flock status in regard to likely immunity status; the placement of 30 sentinel hens per flock and the monitoring of their antibody status was the most useful tool for assessment of flock recovery status. Monitoring of mortality with tracheal, cloacal, and oviduct swabs via commercial Directigen Flu A test with subsequent virus isolation in 9–11-day-old embryonated chicken eggs was additionally helpful as the re-emergence of a latent influenza infection in premise 20 was detected.

The onset of clinical disease for premise 12 (1 and 2) was June 26, 1997; at 15 weeks (October 15, 1997) no antibody was detected in eggs (Table 4). Clinical onset of disease in premise 13 was July 10, 1997. Serological presence of antibody was not found in a September 8, 1997, egg sampling but was noted at 76% of eggs sampled 2 weeks later on September 24, 1997. Serum antibody was first detected on August 26, 1997, with 10% of samples positive. At 6 weeks (November 7, 1997), 12% of eggs sampled had antibody. These data suggest eggs can be used as a monitor for this LPAI infection on a flock basis but for a limited period relatively early in the infection. Eggs may not have detectable antibody in the first few weeks following infection.

These findings leave many questions as to what is an adequate sample size of birds selected and whether random cloacal swabbing is a sensitive method of detecting AI virus from clinically normal appearing hens. Historical questions of sensitivity of tests and appropriateness of specific tests are certainly worthy when new tests or applications of nonspecies tests are used (e.g., human Directigen Flu A Test Kit). Furthermore, the critical question will become "When is it plausible to release quarantine on flocks exposed and infected with LPAI viruses when traditional methods of rapid destruction of infected, suspect, and exposed flocks are not realized?"

Mice are not natural reservoirs of influenza viruses (10), but the presence of wild mice on farms experiencing AI outbreaks and the ability to infect laboratory mice with AI viruses raised concerns about mice being potential vectors during field outbreaks of AI in poultry. Most AI viruses replicate poorly in laboratory mice until adapted by repeated mouse passage. In addition, the strain of mouse is important in producing AI viral infections and disease. Most laboratory strains of mice are genetically susceptible (*Mx1*–) to mouse-adapted influenza viruses, while a few laboratory strains, recently derived from wild mice, are genetically resistant (*Mx1*+) to laboratory infections with influenza viruses (4). Interestingly, in the initial experimental studies (8), as well as our current study, the H5N1 HPAI viruses isolated from poultry and humans in Hong Kong during 1997 did not require adaptation to influenza-susceptible laboratory mice (*Mx1*–) to produce severe clinical signs and death. Furthermore, HK/156 AI virus replicated to high titers in influenza-resistant *Mx1*+ mice, resulting in clinical signs, severe lesions in the respiratory tract, and death.

In the current study, we examined wild caught mice from farms with chickens infected with H7N2 LPAI viruses. AI virus was not recovered from any of the pools of mice sampled. Experimental studies with two H7N2 AI viruses in influenza-susceptible mice (*Mx1*–) demonstrated replication of AI viruses in the respiratory tract and production of infrequent mild lesions. Replication was most extensive with A/chicken/Pennsylvania/19241/97 (H7N2). However, AI virus was not recovered from the influenza-resistant *Mx1*+ mice and lesions were rare and minimal. This suggests wild mice that are genetically more similar to *Mx1*+ laboratory mice were not involved as biological vectors of the H7N2 LPAI virus on poultry farms.

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